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A comparison of tomato (*Lycopersicon esculentum*) lectin with its deglycosylated derivative

David C. KILPATRICK,* Catherine GRAHAM,* Stanislaw J. URBANIAK,*§
Christopher E. JEFFREE† and Anthony K. ALLEN‡
**Regional Blood Transfusion Centre, The Royal Infirmary, Lauriston Place, Edinburgh EH3 9HB, Scotland, U.K., †Department of Botany, King's Buildings, Mayfield Road, Edinburgh EH9 3JH, Scotland, U.K., and ‡Department of Biochemistry, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF, U.K.*

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A deglycosylated derivative of tomato (*Lycopersicon esculentum*) lectin was prepared with the use of trifluoromethanesulphonic acid. Its properties were generally similar to those of the native lectin, but differences were evident in terms of relative agglutinating activity towards sheep, (untreated) human and trypsin-treated human erythrocytes. The two forms of tomato lectin were used in conjunction with a battery of specific antisera to investigate structural relatedness among solanaceous lectins. Immunological cross-reactivity between tomato, potato and *Datura* lectins depends on the integrity of the glycosylated region of those lectins; that between *Datura* lectin and other seed lectins, however, has a separate structural basis.

The function(s) of the carbohydrate moiety of glycoproteins is poorly understood. Eylar (1966) suggested that saccharides serve as labels indicating an extracellular destination, but this is unlikely to be the sole biological purpose of protein glycosylation (Winterburn, 1972). Carbohydrate structures may well have different functions on different glycoproteins, and suggested functions include the enhancement of stability (Pazur *et al.*, 1970; Strumeyer & Malin, 1970) and a role in cell recognition (Hughes, 1975).

In this context, the solanaceous lectins are a particularly intriguing group of glycoproteins, since they have an unusually high carbohydrate content and other properties that distinguish them as a group from other lectins (Kilpatrick, 1983). The properties of potato (*Solanum tuberosum*) lectin after deglycosylation have been studied, but no major differences in characteristics were found from those of its native precursor (Desai *et al.*, 1983). In the present paper we compare the deglycosylated lectin of tomato (*Lycoperisicon esculentum*) with its glycosylated native form and report changes in the nature of the haemagglutinating activity that result from deglycosylation. The deglycosylated lectin of tomato (*Lycopersicon* conjunction with specific anti-lectin sera to investigate structural similarities within this related group of glycoproteins.

§ Present address: Blood Transfusion Service, Aberdeen Royal Infirmary, Foresterhill, Aberdeen AB9 2ZW, Scotland, U.K.

Materials and methods

Saccharides

A mixture of *N*-acetylglucosamine oligomers was prepared as previously described (Kilpatrick & Yeoman, 1978), and diluted to give a suitable inhibitory activity for comparative inhibition studies.

NN'N''-Triacetylchitotriose was isolated with the aid of an ethanol gradient (Rupley, 1964).

Lectins

Tomato lectin was prepared from fruits purchased at a local supermarket by the chromatofocusing method described by Kilpatrick *et al.* (1983). *Datura stramonium* lectin was prepared from seeds by affinity chromatography on ovomucoid–Sepharose (Desai *et al.*, 1981). (*D. stramonium* plants were grown in an University of Edinburgh Department of Botany greenhouse.) The lectin from potato fruits was prepared as previously described (Kilpatrick, 1980), and that from potato tubers in the same way after initial extraction of grated tubers in 3M-acetic acid and precipitation by $(NH_4)_2SO_4$ at 55% saturation

(Matsumoto *et al.*, 1983). The potato-fruit and -tuber lectins formed a line of identity when placed in adjacent wells and subjected to immunodiffusion against antisera raised against the tuber lectin. Homogenates of tomato and sweet-pepper (*Capsicum annuum*) seeds were prepared as previously described (Kilpatrick *et al.*, 1980).

Deglycosylation of tomato lectin

This was carried out for 3 h on ice with the use of trifluoromethanesulphonic acid and anisole (both obtained from Aldrich Chemical Co.) as described by Edge *et al.* (1981).

Immunodiffusion

Ouchterlony double diffusion was performed in a 1% (w/v) agarose gel in a phosphate-buffered saline (Dulbecco A medium from Oxoid) containing 0.05% (w/v) NaN_3. The preparations of rabbit antisera to tomato lectin (Kilpatrick *et al.*, 1980), potato lectin (Ashford *et al.*, 1982) and *Datura stramonium* lectin (Kilpatrick *et al.*, 1979) have been described elsewhere.

Electrophoresis

Electrophoresis in the presence of sodium dodecyl sulphate was performed in homogeneous 10% polyacrylamide gels (Weber & Osborn, 1969) or in commercial gradient gels (Pharmacia PAA 4/30) under conditions previously described (Kilpatrick *et al.*, 1983). Gels were stained for protein with Coomassie Brilliant Blue (Weber & Osborn, 1969) or for carbohydrate with periodic acid–Schiff reagent (Glossman & Neville, 1971) or thymol/H_2SO_4 (Racusen, 1979).

Assay

Protein was determined by the Folin method of Lowry *et al.* (1951), with bovine serum albumin (Sigma, type V) as standard. Carbohydrate (neutral sugar) was determined as described by Dubois *et al.* (1956), with glucose as standard. Lectin activity (Kilpatrick & Yeoman, 1978) was routinely assessed with fresh untreated human erythrocytes. Modifications included the use of glutaraldehyde-fixed (Kilpatrick *et al.*, 1979) and trypsin-treated human erythrocytes. The latter were prepared by incubating a 25% (v/v) suspension of cells in Dulbecco A medium with trypsin (Sigma, type III) at a concentration of 0.2% (w/v) for 5 min at 37°C before washing them extensively.

Results

Treatment of tomato lectin with trifluoromethanesulphonic acid resulted in a quantitative recovery of protein in a product that contained less than 5% carbohydrate as determined by the phenol/H_2SO_4 method; by the same procedure the native lectin was determined to contain 50% carbohydrate. Both deglycosylated and native lectins were subjected to electrophoresis in the presence of sodium dodecyl sulphate in homogeneous and in gradient polyacrylamide gels. Both native and deglycosylated tomato lectin preparations displayed a single band when stained for protein, but the deglycosylated lectin did not react with either of the two carbohydrate stains employed. Under identical conditions the native lectin stained strongly with periodic acid–Schiff reagent and with thymol/H_2SO_4. The deglycosylated lectin always migrated further towards the anode. After overnight electrophoresis (1600 V·h) in gradient gels, the apparent M_r of the deglycosylated lectin was estimated to be 45000 and that of the native lectin to be 68000.

Haemagglutination

The specific haemagglutinating activity of deglycosylated tomato lectin prepared on several occasions ranged from 3 to 10% of that of the original lectin in our standard agglutination assay with untreated human erythrocytes. When the two forms were compared with the use of sheep erythrocytes, however, the specific activity of the deglycosylated lectin was typically more than 30% of that of the native lectin. When trypsin-treated human erythrocytes (either glutaraldehyde-fixed or freshly prepared) were substituted for untreated cells, the deglycosylated lectin attained a 10-fold increase in specific activity, whereas the specific activity of the native form merely doubled. The relative agglutinating activities of the native tomato lectin towards trypsin-treated human, untreated human and sheep erythrocytes are in the proportions 60:30:1; the corresponding proportions for the deglycosylated derivative were 30:3:1. Representative results are given in Table 1.

Table 1. *Relative haemagglutinating activities of native and deglycosylated tomato lectins*

Results are expressed (to the nearest integer) as specific activities, defined as units of agglutinating activity towards a particular type of erythrocyte per mg of protein. For further details see the Materials and methods section.

	10^{-3} × Specific activity	
Erythrocytes used	Native lectin	Deglycosylated lectin
Sheep	21	9
Human	683	28
Trypsin-treated human	1365	284

Table 2. *Comparison of the general properties of tomato lectin and its deglycosylated derivative*

Both preparations were diluted to give a final concentration of 50 μg of protein/ml. Unless otherwise stated, the diluent used was Dulbecco A medium, pH 7.3, which was also used as the pH-stability control. Each result is expressed as a percentage of an appropriate control.

Treatment	Activity (% of control) Native lectin	Deglycosylated lectin
70°C for 60min	100	100
80°C for 30min	37	37
100°C for 5min	6	6
3.5h at 37°C in 0.1M-glycine/HCl, pH 1.0	100	100
3.5h at 37°C in 0.2M-ethanolamine/HCl, pH 10.0	100	100
Thyroglobulin (1mg/ml)	18	18
(GlcNAc)-oligomer mixture (at suitable dilution)	6	6
NN'N''-Triacetylchitotriose (1mg/ml)	6	6
0.2% Trypsin for 30min at 37°C	50	50
0.2% Pronase for 30min at 37°C	6	6

General properties

The tomato lectin is very stable at low pH and at high temperatures. When the lectin and its deglycosylated derivative were simultaneously exposed to the same range of test conditions, the two forms exhibited identical characteristics (Table 2). They also showed identical susceptibility to inhibition by simple and complex carbohydrates, and identical susceptibility to degradation by proteolytic enzymes (Table 2).

Serological studies

Native and deglycosylated tomato lectin were allowed to react in immunodiffusion plates with rabbit and anti-(tomato lectin) serum, anti-(*Datura* lectin) serum and anti-(potato lectin) serum, as were other lectins and extracts from solanaceous sources. Some of these reactions are illustrated in Fig. 1. Deglycosylated tomato lectin formed a strong precipitation line with anti-(tomato lectin) serum, but not with either anti-(potato lectin) serum or anti-(*Datura* lectin) serum. The native tomato, potato and *Datura* lectins formed a cross-reacting group irrespective of the specificity of the antiserum used. The extracts of tomato or sweet-pepper seeds reacted only with anti-(*Datura* lectin) antiserum.

Both native and deglycosylated forms of tomato lectin were incubated with 0.2% Pronase for 6h at 37°C; this treatment destroyed all haemagglutinating activity. Pronase-treated native lectin reacted in double-diffusion experiments equally well with all three specific antisera. Pronase-treated deglycosylated tomato lectin did not form a detectable line of precipitation with any of the antisera.

None of the preparations referred to in this section reacted with non-immune rabbit serum.

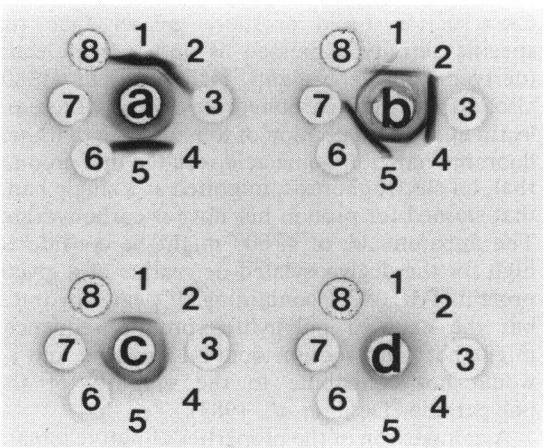

Fig. 1. *Serological reactions of tomato lectin, deglycosylated tomato lectin and related lectins*

Double diffusion was carried out on Gelbond (Miles Laboratories) for 48–72h before the gel was washed free of soluble protein in Dulbecco A medium and dried. The dried gel was stained with Coomassie Brilliant Blue (Weber & Osborn, 1969), then washed in 10% (v/v) acetic acid followed by distilled water. Centre wells were filled with 20 μl of serum: (a) anti-(tomato lectin) serum (from rabbit 'Peter'); (b) anti-(*Datura* lectin) serum; (c) anti-(potato lectin) serum; (d) pre-immune rabbit serum (also from rabbit 'Peter'). Peripheral wells were filled with 15 μl of antigen solution: (1) and (5) tomato lectin (2 μg of protein); (2) deglycosylated tomato lectin (4 μg of protein); (3) and (6) *Datura stramonium* lectin (10 μg of protein); (4) potato lectin (4 μg of protein); (7) homogenate of sweet-pepper (*Capsicum annuum*) seeds; (8) homogenate of tomato seeds.

Discussion

The tomato lectin has an unusually high carbohydrate content. Since less than one-third of the dry weight of the lectin can be accounted for as Folin-reactive protein, the value of 50% carbohydrate, derived from the equal masses estimated for Folin-reactive protein (relative to a bovine albumin standard) and phenol/H_2SO_4-reactive carbohydrate (relative to a glucose standard), might be an underestimate. Consequently, the specific activity of the purified tomato lectin expressed as units/mg of protein (typically about

650×10^3) is 3-fold or more greater than the specific activity expressed as units/mg of lectin (derived from dry weight). Nachbar *et al.* (1980) also estimated the carbohydrate content of tomato lectin at 50%. Incubation of tomato lectin with trifluoromethanesulphonic acid resulted in a product that, on electrophoresis, migrated as a single band that stained for protein but not for carbohydrate. The apparent M_r of 45000 might be considered high for the deglycosylated derivative of a glycoprotein of M_r 68000 containing 50% carbohydrate, but the exposed polyhydroxyproline sequences might not bind sodium dodecyl sulphate and so would not contribute to the mobility of the polypeptide (Desai *et al.*, 1983).

A comparison of the properties of native tomato lectin with its deglycosylated derivative was carried out to obtain insight into the possible function (if any) of the carbohydrate moiety. Few differences were found and, although the tomato lectin provides another example of an association between high thermal stability and relative resistance to proteolysis (Daniel *et al.*, 1982), the carbohydrate moiety does not appear to contribute to either property. The deglycosylated derivative was capable of functioning as a lectin, but exhibited an altered cell specificity in haemagglutination assays. This could be an indication that a glycosylated domain influences the saccharide-specificity of the lectin. However, both native and deglycosylated forms displayed a quantitatively similar susceptibility to inhibition by thyroglobulin or *N*-acetylglucosamine oligomers. Agglutination of cells is a complex phenomenon depending on many factors (Nicolson, 1974), and it is perhaps more likely that the carbohydrate moiety is required to maintain the conformation that permits ready agglutination of untreated human erythrocytes without altering the saccharide-specificity of the lectin. The haemagglutinating properties of the deglycosylated tomato lectin resemble those of the lectin from *D. stramonium*; the latter is also 10 times more active towards trypsin-treated human erythrocytes than towards untreated cells, and does not distinguish between sheep and human erythrocytes, unlike the tomato and potato lectins (Kilpatrick, 1983). It may be significant that the *Datura* lectin has a lower carbohydrate content, estimated at 28% by the phenol/H_2SO_4 method (Horejší & Kocourek, 1978).

Deglycosylated lectins provide an additional tool for the immunochemical analysis of lectin structure. Kilpatrick *et al.* (1980), using an anti-(*Datura* lectin) serum, found cross-reacting material from various species within the Solanacaeae, and in various tissues of the same species. It was proposed that the solanaceous lectins can be placed in two groups, the non-seed lectins (which are readily inhibited by oligomers of *N*-acetylglucosamine and include the best-characterized lectins on this Family), and the seed lectins, which vary in their saccharide-specificities and in their abilities to agglutinate erythrocytes. More recently, evidence has been presented that the immunological cross-reactivity between *Datura*, tomato and potato lectins arises from a common glycosylated region in those molecules (Ashford *et al.*, 1982; Desai *et al.*, 1983). It seemed likely, therefore, that the specific reactions between anti-(*Datura* lectin) serum and extracts from distantly related species (Kilpatrick *et al.*, 1980; Jeffree *et al.*, 1983) might also depend on a similar carbohydrate unit, and this was suggested by Kilpatrick (1983). The results described in the present paper are consistent with the view that tomato (fruit), potato (fruit or tuber) and *Datura* (seed) lectins share a common glycosylated domain, which is the principal basis of the cross-reactions between them. However, it is equally clear that a similar carbohydrate unit does not account for the reactions between pepper-seed or tomato-seed extracts and anti-(*Datura* lectin) serum, since those extracts did not react with anti-(tomato lectin) serum or anti-(potato lectin) serum. Moreover, when tomato-seed homogenate and tomato-fruit lectin were placed in adjacent wells and allowed to react against anti-(*Datura* lectin) serum, lines of non-identity were clearly observed. It appears that the cross-reactivity between the well-characterized *N*-acetylglucosamine oligomer-specific lectins and the cross-reactivity between *Datura* lectin and numerous other solanaceous extracts (which may or may not involve a separate glycosylated region) have a separate antigenic basis, and the original serological division into solanaceous seed and non-seed lectins seems justified.

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